MICROBIOTEST PROTOCOL

TESTING PRE-SATURATED OR IMPREGNATED TOWELETTES FOR TUBERCULOCIDAL EFFECTIVENESS

Prepared for
Professional Disposables International, Inc.
Two Nice-Pak Park
Orangeburg, NY 10962

Testing facility
MICROBIOTEST
105 Carpenter Drive
Sterling, VA 20164

March 5, 2010

OBJECTIVE:

This test is designed to substantiate tuberculocidal effectiveness for impregnated or pre-saturated towelettes, single or multiple uses, to be registered with the Environmental Protection Agency. The test evaluates the effectiveness of products as spot disinfectants for contaminated surfaces. The test is designed to determine the tuberculocidal effectiveness of the test compound by incorporating AOAC methods for the confirmative in vitro test for tuberculocidal activity (DIS/TSS 6) and EPA Efficacy Data Requirements for Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection as well as Health Canada registration requirement aspects.

TESTING CONDITIONS:

A total of ten carriers per lot will be tested using three lots of test agent. The carriers, inoculated with *Mycobacterium bovis,* will be wiped for a specified time directed by the sponsor or label instruction and held for the exposure time and at the temperature specified by the sponsor. The carriers and the liquid from the towelette will be cultured, incubated and observed for visible growth.

MATERIALS:

A. Test agent will be supplied by the sponsor: Disinfectant to be tested - see last page.

The test agent will be tested as supplied by the sponsor unless written instructions direct otherwise. All operations performed on the test agent such as dilutions or specialized storage must be specified by the sponsor before the initiation of testing.

The sponsor assures MICROBIOTEST testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MICROBIOTEST will retain all unused test agents for a period of at least three months after completion of the study, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MICROBIOTEST including but not limited to:

1. Challenge organism, required by AOAC: *Mycobacterium bovis* (BCG). Organon Teknika, Corp.

2. Media and reagents:
   a. Middlebrook 7H11 agar
   b. 0.85% NaCl containing 0.1% Polysorbate 80 (SS+)
   c. Modified Proskauer-Beck Medium (MPBM)
   d. Neutralizer: DE or MPBM containing appropriate neutralizers.
   e. Middlebrook 7H9 broth (7H9)
   f. Kirchner medium (KM)
   g. Phosphate buffer saline solution (PBS)
   h. PBS containing 1% Polysorbate 80 (PBS+)
   i. Saline solution
   j. Heat-inactivated fetal bovine serum (FBS) – if requested

3. Laboratory equipment and supplies

4. Carriers: specified by sponsor-see last page

TEST SYSTEM IDENTIFICATION:

All tube supports, baskets, or other culture-containing devices will be labeled with the following information: microorganism, test agent and project number.

EXPERIMENTAL DESIGN:

A. Preparation of inoculum:

From stock culture, tubes containing 20 mL of MPBM will be inoculated with *M. bovis* and incubated in a slanted position at 37±2C while remaining quiescent for 21–25 days.

For each culture, all growth will be harvested from the surface of the 20 mL culture and transferred into a sterile tissue grinder. One mL of SS+ will be added and the culture will be macerated to break up large clumps. Nine mL of MPBM will be added to the homogenized culture.

The homogenized suspension will be transferred to a sterile tube and the culture will be allowed to settle for 10-15 minutes. Using a sterile pipette, the culture that remains in suspension will be transferred to a clean, sterile flask.

If requested, heat-inactivated FBS will be added to the culture to yield a 5% organic load.

If necessary, the suspension will be diluted with MPBM until a spectrophotometric reading appropriate to achieve at least 10^6 colony-forming units CFU) per carrier at 650 nm is achieved.

B. Carrier preparation:

The carriers will be sterilized by placing slides in evaporating dishes matted with two pieces of filter paper, heating them in a hot air oven for two hours at 180C, cooling and storing them at room temperature until use.

C. Carrier inoculation:

Aliquots (0.01 mL – 0.03 mL) of the inoculum will be individually transferred onto one sq inch areas on the sterile carriers and spread uniformly over the entire area with a glass rod. Each dish will be covered and the operation will be repeated for the rest of the carriers. Carriers will be dried for 30 minutes at 37±2C.

D. Test agent preparation:

The test agent will be used as received by the sponsor of the study. The canisters or packet(s) containing the towelettes will be inverted or mixed to re-saturate the towelettes.

E. Test:

For each lot of test material, ten carriers will be treated using the contact time stipulated by the sponsor. When using towelettes from a new canister, the lid will be removed and the center of the roll pulled out and inserted into the lid opening. The lid will be replaced and the towelette pulled through the lid opening, making it ready for use in testing. When using individually wrapped towelettes, the towelette will be confirmed for moistness to touch and then folded as described below.

Initially the towelette will be folded lengthwise twice and then folded five times inward beginning from the far end. Then the outside edges will be pulled upward to form a "u" shape and grasped preferably on one side with the thumb and on the other side with the index and middle finger. The folded towelette will be rotated 90°.

Each contaminated carrier will be wiped using two complete horizontal strokes, with one right to left and back to right considered as one stroke; and then wiped using two complete vertical strokes, with one up to down and back to up considered one stroke for a total of four complete strokes. The used end will be flipped upward towards self, reoriented appropriately and then used to wipe the next carrier. The next three carriers will be wiped in a similar fashion - the used portion will be folded up-and-over each time. Once five carriers have been wiped, the towelette will be unraveled.

The second lengthwise fold will be unfolded and refolded in the opposite direction. The towelette will be refolded five times as before and the above procedure for wiping the first five carriers will be repeated for wiping the last five carriers. This process will be repeated until a total of ten carriers have been wiped with one towelette.

Each carrier will be transferred into 20 mL of neutralizer at the completion of the specified contact time and thoroughly shaken.

After at least ten minutes in the neutralizer, each carrier will be shaken and transferred to a tube containing 20 mL of MPBM. From each tube of neutralizer, two mL will be subcultured to a tube containing 20 mL 7H9 broth and 2 mL will be subcultured to a tube containing 20 mL KM. Each subculture tube will be shaken and the sequence will be repeated for all carriers.

F. Controls:

1. Viability controls:

Two contaminated carriers each will be added to tubes containing 20 mL MPBM, 20 mL 7H9, and 20 mL Kirchner's. The tubes will be incubated with the test in order to confirm the ability of the recovery media to support growth of the challenge microorganism.

2. Neutralizer effectiveness:

A single carrier will be exposed to the test material and processed in the same manner as the test carriers. To each tube of 20 mL 7H9, 20 mL Kirchner's and 20 mL MPBM media, fewer than 100 CFU will be added. The concentration of the bacterial suspension inoculated into these tubes will be confirmed by filter plating duplicate aliquots on 0.45 µm membrane filters using Saline solution. The filters will be placed onto 7H11 agar plates and the plates will be incubated for 14 – 25 days at 37±2C.

3. Sterility controls:

Two mL of neutralizer will be added to a tube of MPBM, a tube of 7H9 and a tube of KM. The tubes will be incubated with the test in order to demonstrate the sterility of the media used in the study.

4. Carrier counts:

Each of three carriers will be placed in 20 mL of PBS + 1% Polysorbate 80 and subjected to ultrasound for 5 minutes. Ten fold serial dilutions will be conducted in PBS and one mL aliquots from selected dilutions will be filtered using Saline solution onto 0.45 µm membrane filters in duplicate. The filters will be placed onto 7H11 agar plates and the plates will be incubated for 14 – 25 days at 37±2C.

G. Incubation:

All tubes used for secondary transfers (MPBM, 7H9, and KM) and all viability, neutralizer effectiveness, and sterility control tubes will be incubated for 60 days at 37±2C and the results will be reported as growth or no growth. If no test culture tube shows visible growth, the test (and control) tubes will be incubated an additional 30 days before the final reading is made. All plates will be incubated for 14-25 days at 37±2C.

H. Confirmation of challenge microorganism:

On the day of the final reading, acid-fast stains will be performed for all test culture tubes demonstrating visible growth and two viability control tubes. In addition, the growth morphology of the microorganism will be observed. This will serve to confirm use of the correct challenge microorganism.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance. There are no proposed statistical methods for this test.

- The carrier counts must be at least 1×10^6 CFU/ carrier
- The neutralizer must be effective

PRODUCT EVALUATION CRITERIA:

The compound meets effectiveness requirements if no visible growth occurs in any replicate tube, for any of the subculture broths.

DATA PRESENTATION:

The final report will include the following information (if appropriate):

- The number of positive carriers.
- Results of all of the controls.

CONFIDENTIALITY:

All data generated at MICROBIOTEST are held in strictest confidence and are available only to the sponsor. In turn, no reference to the work, data, or MICROBIOTEST may be made public without the written consent of MICROBIOTEST.

REPORT FORMAT:

MICROBIOTEST employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification
- Test agent identification
- Type of assay and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria
- Signed Quality Assurance and Compliance Statements

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately. The date the study director signs project sheet number one will be the initiation date. All project sheets will be forwarded to the study sponsor.

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor:

A. Name and address: Professional Disposables International, Inc.
Two Nice-Pak Park
Orangeburg, NY 10962

B. Test agent: PDI SANI-CLOTH BLEACH WIPES
Active ingredient: 0.54% ACTIVE SODIUM HYPOCHLORITE
Lot 1: AE-1086-756-038
Lot 2: AE-1086-756-039
Lot 3 AE-1086-756-040

Exposure time: 2 minutes (must be ≤ 10 minutes)

Exposure temperature: Ambient room temperature (20±1C)

C. Organic load – serum added to achieve 5% in the inoculum: ☒ yes ☐ no

D. Precautions/storage conditions: refer to MSDS or certificate of analysis
☒ provided ☐ not provided

REPORT HANDLING:

The sponsor intends to submit this information to (CHOOSE ONE ONLY):
■ EPA ☐ FDA ■ Health Canada ☐ CAL DPR
☐ ARTG ☐ Other: _____

STUDY CONDUCT: ■ GLP ☐ non-GLP

PROTOCOL APPROVAL:

Sponsor: Phyllis Vitolo Date: 3/10/10

Printed Name: Phyllis Vitolo

Date Issued: 03/25/10 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 735-102

STUDY TITLE: TESTING PRE-SATURATED OR IMPREGNATED TOWELETTES FOR TUBERCULOCIDAL EFFECTIVENESS	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 03/25/10 Date		
TEST AGENT(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-038	03/12/10	A183
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-039	03/12/10	A184
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-040	03/12/10	A185
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITION:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator		

PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ☐Liquid ☐ Aerosol ■ Other: Towelettes with liquid

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 03/25/10 **TERMINATION DATE:** 06/23/10

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Health Canada

SPONSOR: Professional Disposables International, Inc. Two Nice-Pak Park Orangeburg, NY 10962	**CONTACT PERSON :** Phyllis Vitolo Tel. No. 845-365-1700 x500 E-mail: pvitolo@nicepak.com

TEST CONDITIONS:

Challenge organism(s):	*Mycobacterium bovis* (BCG) Organon Teknika, Corp.
Active ingredient(s):	0.54% Active Sodium Hypochlorite
Neutralizer(s):	DE Neutralizing Broth
Contact Time(s):	Two minutes
Contact Temperature(s):	Ambient Room Temperature (20±1C)
Dilution(s):	Not applicable – ready to use test agent
Serum:	■ Yes / ☐ No (HI fetal bovine serum added to achieve 5% concentration)
Incubation Time(s):	60 days plus 30 days if test is negative for growth (test and controls) 14-25 days (control plates)
Incubation Temperature(s):	37±2C
Comments:	Not applicable

Date Issued: 06/23/10 Project Sheet No. 2	Page No. 1 Laboratory Project Identification No. 735-102		
STUDY TITLE: TESTING PRE-SATURATED OR IMPREGNATED TOWELETTES FOR TUBERCULOCIDAL EFFECTIVENESS	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 06/28/10 Date		
TEST AGENT(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-038	03/12/10	A183
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-039	03/12/10	A184
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-040	03/12/10	A185
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITION:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator		
CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Health Canada			
SPONSOR: Nice-Pak Products, Inc. 711 Executive Boulevard – Suite P Valley Cottage, NY 10989	**CONTACT PERSON :** Phyllis Vitolo Tel. No. 845-365-1700 x500 E-mail: pvitolo@nicepak.com		

EXPLANATION:

Protocol Amendment(s)

1. Page 3 of the protocol states the carriers will be "specified by the sponsor-see last page". The sponsor did not specify on the last page of the protocol. However per standard practice; glass microscope slides (1" x 3" with a 1" x 1" surface for contamination and treatment) were used for testing. This amendment is made to correct the protocol.

2. The sponsor address changed. The new address is outlined above.

Date Issued: 07/16/10 Project Sheet No. 3 Page No. 1 Laboratory Project Identification No. 735-102

STUDY TITLE: TESTING PRE-SATURATED OR IMPREGNATED TOWELETTES FOR TUBERCULOCIDAL EFFECTIVENESS	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 07/16/10 Date		
TEST AGENT(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-038	03/12/10	A183
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-039	03/12/10	A184
PDI SANI-CLOTH BLEACH WIPE	AE-1086-756-040	03/12/10	A185
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITION:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator		

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Health Canada

SPONSOR: Professional Disposables International, Inc. 711 Executive Boulevard – Suite P Valley Cottage, NY 10989	**CONTACT PERSON :** Phyllis Vitolo Tel. No. 845-365-1700 x500 E-mail: pvitolo@nicepak.com

EXPLANATION:

Protocol Amendment(s)

3. The identification of the sponsor was incorrectly listed on Project Sheet No. 2 as well as the final report as Nice-Pak Products, Inc. An amended final report will be issued to reflect the correct name.